PUBLIC HEALTH REPORTS.

PLAGUE INVESTIGATIONS IN INDIA.

The following extracts and condensations are from the reports on plague investigations in India issued by the advisory committee appointed by the secretary of state for India, the Royal Society, and the Lister Institute, and published in the Journal of Hygiene, September, 1906, and July, 1907:

Transference of plague from rat to rat.—In experiments which were a repetition of those of Gauthier and Raybaud (1902, 1903) it was shown that, in the presence of the common Indian rat flea (*Pulex cheopis*, Rothschild), plague may be spread from a plague-infected rat to a healthy rat confined in close proximity, but in such a way as to prevent contact with the body or excreta of the sick rat.

Two wire cages were placed in a glass box, the cages rising above the level of the top of the box, the intervening space being bridged over by a fine variety of muslin (tulle), which is impervious to fleas. Each cage stood in a tiny tray, which collected the urine. It was found necessary to fill both trays with either dry earth or sand in order to provide dryness and shelter for the fleas, which without some such cover rapidly died out. Each cage was provided with a lid through which the rats were introduced and food and water given to them. The lids when put on were also covered with fine muslin.

Rats placed in the cages were therefore protected against invasion from outside the whole apparatus by particles larger than such as are capable of passing through fine muslin; they could not come in contact with the bodies, feces, or urine of each other.

In one cage was placed a rat inoculated with plague, together with 10 to 20 fleas obtained from Bombay rats. More fleas were in some experiments added subsequently. As soon as the inoculated rat was found dead, a healthy rat was placed in the other cage. The dead rat was left in the first cage till eight to twelve hours later, when it was removed and examined for septicemia. If no plague bacilli were found in the blood microscopically, the experiment was abandoned.

If the healthy rat died, a careful post-mortem examination was made, the position of the buboes noted, and smears from the bubo, spleen, and heart's blood stained and examined. Further, cultures were made from the liver and heart's blood, and the resulting growths tested both culturally and on animals, a number of rats being inoculated with each strain. All rats which had not died in three weeks from the day of being first placed in the second cages were killed with chloroform and examined, to make certain that they were not suffering from a subacute or chronic form of plague. In these cases

also, if there was any doubt in the matter, cultures were taken and animal tests employed.

As a result of these experiments, healthy rats on 30 occasions contracted plague in sequence to living in the neighborhood of plague-infected rats under circumstances which prevented the healthy rats coming in contact with either the body or excreta of the plague-infected rats. In all cases a fairly abundant supply of fleas was present; these could pass freely between the two rats used in each experiment, and, except for "aerial contagia," formed the only apparent means of communication between the animals. The presumption is that plague was transferred from the sick to the healthy rat by the agency of the fleas.

To exclude aerial infection, attempts were made to conduct a similar series of experiments in the absence of fleas. This was, however, found to be impracticable, because even with the greatest care it was impossible to insure that the animals were entirely free from fleas. Experiments were therefore instituted in which the fleas themselves were taken from a sick rat and placed on a healthy animal.

Another set of experiments was made to demonstrate the transference of plague from rat to rat by transference of fleas. Bombay rats were inoculated with a virulent culture of *Bacillus pestis*, placed separately in flea-proof cages and supplied with rat fleas. In the event of any of these rats dying with plague and if their blood contained any bacilli on microscopical examination, the fleas were caught and transferred to a fresh flea-proof cage in which was placed a healthy rat. The flea supply for each healthy rat was always obtained from two or more septicemic rats. As in the first series of experiments a careful post-mortem examination was made of all uninoculated rats which died. The position of the buboes was noted; smears from the bubo, spleen, and heart's blood were stained and examined, and cultures were made from the liver and heart's blood. The resulting growths were tested both as regards their cultural and animal reactions.

All rats which had not died after three weeks were killed with chloroform and examined.

In 21 experiments out of 38 (55 per cent), healthy rats living in flea-proof cages contracted plague in sequence to receiving fleas collected from rats dead or dying of septicemic plague in another cage. The possibility of the rat flea (*Pulex cheopis*) carrying plague from one rat to another was therefore demonstrated directly.

Experimental production of epidemics among guinea pigs.—Experiments which had as their object the determination of the relative importance of the Indian rat flea, *Pulex cheopis*, and of actual close contact in the absence of fleas in the dissemination of plague from animal to animal, were carried out in a series of six small "go-downs" or cabins built especially for this purpose.

Guinea pigs and also two monkeys were used for these experiments, as it had been shown by Liston (1905) that the rat flea that has to be dealt with in India readily attacks these animals in the absence of its natural host.

The godowns were six in number and built in a row. The walls, 9 inches thick, were built of brick and mortar, while the floors were of concrete on the top of a high plinth. The walls and floors were

therefore impervious to rats. Each go-down was entered by a tightly fitting wooden door. The door opened inward into an inspection chamber separated from the rest of the interior by means of wire netting, carefully fitted onto a wooden framework and extending up to a wire netting which covered the inside of the roof. This netting, as also that under the roof, was made of stout wire and had a mesh of half an inch. A door, also of wire netting, gave access from the inspection chamber into the interior of the go-down.

In certain of the go-downs, with the exception of the doors, there was no opening in any of the walls. In certain others there were, for the purpose of ventilation, small windows, measuring 1 foot square, in the front wall. These windows were closed by a double layer of wire netting carried on a wooden framework let into the masonry of the wall. The essential difference in the construction of the go-downs consisted in the structure of the roofs. Two had roofs of ordinary country tiles, placed in four layers on the top of wooden laths. Immediately on the inside of this roof there was a wire netting carried on a wooden framework, the framework being carefully built on all sides into the masonry of the walls. In the roof (a country-tiled roof) of one go-down a certain amount of light was allowed to penetrate through a small glass window in the tiles. This opening did not exist in the roof of another go-down having a country-tiled roof. In the former of these two go-downs there was a second wire screen 10 inches below the one immediately under the tiles. This screen was also carried on a wooden framework built into the walls. While, therefore, rats could inhabit the tiles of the roof and build their nests there, they were completely shut off, in one case by a double screen of wire, in another by a single screen of wire, from the interior of the go-downs. The object of the double screen of wire was to prevent any possible contact between experimental animals in the go-down and the wild rats inhabiting the roof.

The roofs of two other go-downs differed from those just described in having for a roof a single layer of flat Mangalore tiles instead of country tiles. In one of the go-downs with a Mangalore-tile roof there was a ventilator, also of Mangalore tile, through which a small amount of light was allowed to penetrate into the interior of the go-down, the ventilator not existing in the other go-down with a similar roof. Further, the roofs of both these go-downs were separated from the interior by a single layer of wire netting. Mangalore tiles, in comparison with country tiles, afford a poor shelter to rats. It was found, therefore, that the rats inhabiting the roofs of go-downs with Mangalore-tiled roofs were not nearly so numerous as in the case of go-downs with country-tiled roofs. That rats inhabit the roofs of these go-downs has often been a subject of observation. They were frequently seen on the top of the wire during the day while the go-downs were being examined. As many as twelve rats were seen in a single go-down at one time. The species seen was always *Mus rattus*. Further, rats' nests were found on several occasions on the wire netting. Rat dung was also found in the go-downs themselves.

The roofs of the last two of the series of six go-downs was made of a single layer of corrugated iron fastened down with cement to the top of the walls all around. It is evident, therefore, that no rat

could penetrate inside the roof of either of these go-downs. The wire netting under the roof of one of these go-downs was of a single layer; in the case of the other there was a double layer.

The fact is emphasized that the only essential difference in the six go-downs was the structure of the roofs. This difference, however, was of such a nature that the natural supply of fleas, depending as it did on the number of rats inhabiting the roofs, and the amount of light, varied in the different go-downs. In the case of the first two go-downs, the roofs of which offered good protection and shelter to the wild rat of Bombay, the flea supply was abundant and regular. In the case of the second pair of go-downs, the roofs of which offered only poor protection to rats, the flea supply was more or less scanty; while in the last two go-downs, the roofs of which were absolutely impervious to rats, no fleas were able to gain access unless carried in though the door on the experimental animals themselves, or by the attendant when feeding these animals.

All animals dying in the course of the experiments were submitted to a careful detailed post-mortem examination. This examination included a microscopical examination of smears of the bubo, of the spleen, and of the heart's blood. Further, cultures were taken, and if there was the slightest doubt of the diagnosis, cultural and animal inoculation tests were made.

Using these go-downs, experiments were conducted in which epidemic plague did not occur when healthy guinea pigs lived in close contact with plague-infected guinea pigs under conditions where access of fleas was prevented, but in which, under otherwise similar conditions, plague spread among the healthy animals in places where fleas were abundantly present. Simond, Gauthier and Raybaud, and Liston never succeeded in infecting animals from one another when healthy and plague-infected animals were confined together in the same cage, if fleas were excluded and the animals were not allowed to devour the cadavers of their dead comrades. The experiments of the committee were conducted upon a somewhat larger scale than had been previously possible. By experiment an epidemic in an uninfected go-down was started by the transference of fleas from guinea pigs dying from plague. The epidemic was maintained by the introduction of fresh fleas. It was also indicated by experiments that when an epidemic has occurred among a number of guinea pigs the contagion still remains in the place, and is effective in proportion as the test animals are accessible to and found to be infested with fleas. It was shown that fleas removed from infected guinea pigs, and isolated in test tubes, can convey plague to healthy animals on which they are allowed to feed; and also that plague is not conveyed from mother to offspring in the absence of fleas. Finally, a monkey was safely exposed in a plague-infected place where the free access of fleas to his person was prevented, whereas his companion, not so protected, succumbed.

The following conclusions were drawn as a result of the experiments cited above:

Close contact of plague-infected animals with healthy animals, if fleas are excluded, does not give rise to an epizootic among the latter. As the go-downs were never cleaned out, close contact included contact with feces and urine of infected animals, and contact

with and eating of food contaminated with feces and urine of infected animals, as well as with pus from open plague ulcers.

Close contact of young, even when suckled by plague-infected mothers, does not give the disease to the former.

If fleas are present, the epizootic, once started, spreads from animal to animal, the rate of progress being in direct proportion to the number of fleas present. The epizootic was very rapid in those go-downs in which the flea population was abundant and was kept up by a natural supply from the roof; it was much slower in a go-down in which the flea supply was kept up artificially; and, finally, it was slowest of all in a go-down in which there was no definite natural supply of fleas, and from which the fleas were daily removed for a period of six days, after which removal only a comparatively small number could be caught.

An epizootic of plague may start without direct contact of healthy animals and infected animals. Thus, in one experiment healthy guinea pigs were not put in the go-down until the last inoculated guinea pig formerly kept therein had died and been removed.

It having been shown by direct experiment that the rat flea can convey plague from rat to rat, experiments of a similar nature with the fleas removed from infected go-downs were recorded.

Infection can take place without any contact with contaminated soil. In certain of the experiments the guinea pigs placed in wire cages 2 inches above the ground developed plague. In another experiment a monkey placed in a go-down, but never in contact with the ground, also developed plague.

Aerial infection was excluded. Thus, guinea pigs suspended in a cage 2 feet above the ground did not contract the disease, while in the same go-down those animals allowed to run about and those placed 2 inches above the floor became infected. Further, a monkey surrounded by " tangle foot " escaped, though exposed as much to aerial infection as the control animal which contracted the disease.

Experiments in plague houses in Bombay.—Observations were made which go to prove, both indirectly and directly, that in a plague-infected house the infection may be due to the presence therein of rat fleas, which are capable of transmitting the disease to animals.

In choosing the houses for this present purpose care was taken to insure that they were really plague infected. Thus for the most part only those rooms were used in which two or more people were suffering from the disease, or in which rats infected with plague had been found, or in which there was a history of dead rats having been discovered. In some cases, but not in all, there was absolutely no doubt about the house being infected. A careful post-mortem examination was made of every animal which died. The naked eye post-mortem appearances were first recorded; then smears of the spleen, of the heart blood, and of the bubo, if a bubo was present, were stained and examined. Finally, cultures were taken from the organs and from the heart blood. These cultures were tested as to their appearance on agar and as to their power of forming involution forms on salt agar and stalactites in oil broth. Further, subcultures of every strain were inoculated into a number of wild Bombay rats, which rats were examined post-mortem for signs of plague and for the presence of the plague bacillus in smears of the spleen.

The following is a summary of the observations and conclusions of the committee as a result of experiments with plague houses reported in 1906:

Guinea pigs allowed to run free in plague houses in many instances attracted a large number of fleas, which fleas were mostly rat fleas. Of these animals, 29 per cent contracted plague and died from the disease. The position of the bubo in the great majority of these cases was cervical.

If a plague house had been previously disinfected by the ordinary means of disinfection, fleas were still caught in large numbers on guinea pigs set free in them. Further, a considerable number (29 per cent) of these animals died of plague, the bubo in the great majority of these cases being in the cervical region.

Fleas transferred from plague-infected rats found dead or dying in houses were able to transmit plague to healthy animals in flea-proof cages in the laboratory. The bubo in all cases was in the cervical region.

Fleas transferred from guinea pigs and other animals which had been placed for a few hours in plague houses were able to transmit the disease to fresh animals when fed on these in flea-proof cages in the laboratory. The situation of the bubo in these animals was in the great majority of cases in the cervical region.

Animals were placed in plague houses in pairs both protected from soil and contact infection and both equally exposed to aerial infection, but one protected from fleas by means of a fine metallic curtain and the other not so protected. None of the protected animals contracted plague while several of the unprotected animals died of the disease. The position of the bubo in every instance was in the cervical region.

Animals were placed in plague houses in pairs, both protected from soil and contact infection and both equally exposed to aerial infection, but one surrounded with a layer of "tangle foot" and the other surrounded with a layer of sand. Many fleas were caught on the "tangle foot," a certain proportion of which were found on dissection to contain in their stomachs abundant bacilli microscopically identical with plague bacilli. Out of 85 human fleas dissected only one contained these bacilli, while out of 77 rat fleas 23 were found thus infected. The animals surrounded with "tangle foot" in no instance developed plague, while several (24 per cent) of the nonprotected animals died of the disease.

During the epidemic of 1907 previous observations were confirmed and amplified and the following conclusions reached:

In 19 out of 100 experiments guinea pigs allowed to run free in houses which were presumably plague infected developed the disease and died. On three occasions—namely, on every occasion on which the experiment was made—fleas transferred from plague-infected rats found in houses infected fresh guinea pigs in the laboratory. Rat fleas caught on guinea pigs in plague houses and transferred to fresh guinea pigs transmitted the disease in 35 per cent of the cases. A census of rat fleas in houses in Bombay which were proved plague infected indicated that rat fleas were twelve times as numerous as in control houses, and that in presumably plague-infected houses rat fleas were four times as numerous as in control houses. In 41 out of

130 fleas taken on guinea pigs in plague-infected houses bacilli microscopically indistinguishable from plague were found in the stomach. In the case of 24 of the 27 houses definitely proved to be plague infected, dead rats had been found shortly before the experiments were made.

Plague bacillus in rats.—Interesting observations were made on the number of plague bacilli in the blood, urine, and feces, respectively, of rats which had died of plague.

The blood of plague-infected rats may contain an enormous number of plague bacilli, even as many as 100,000,000 per cubic centimeter having been found before death. On the other hand, rats occasionally die from plague with little or no septicemia.

While the blood of a rat may have as many as 100,000,000 organisms in a cubic centimeter, the urine may have none at all, or at least less than 10 per cubic centimeter. Plague bacilli were discovered in the urine in 29 per cent of the cases. When the urine contained plague bacilli they were always present in much fewer numbers than in the blood.

The feces of rats dead of plague even when the blood contains abundant bacteria, are not, it was concluded, highly infective and would appear to play little part in the spreading of the epizootic.

Plague bacillus in man.—Observations were also made on the quantitative estimation of the septicemia in human plague, involving an investigation of the blood of 28 patients suffering from plague, with an examination of 74 specimens. The *Bacillus pestis* was not found in the blood of 5 patients whose illness ended in recovery; nor was it found in 7 of the fatal cases. The salient facts ascertained from an analysis of the remaining 16 fatal septicemic cases may be recapitulated thus: Microscopical examination of the blood can not be regarded as a trustworthy criterion of the degree of septicemia; a severe septicemia may be present at a comparatively early stage of the disease and for a considerable number of hours before death, and the septicemia may be of an irregular or fluctuating type.

The diagnosis of natural rat plague.—Cases of plague in rats, like human cases, may be divided into two classes, according as to whether or not a bubo is present. The bubo, if present, is the most important diagnostic sign of plague. Of other characteristic appearances, those occurring in the liver of plague-infected rats are of primary importance from the point of view of diagnosis. Hemorrhages in various parts of the body are commonly met with, and an abundant clear pleural effusion constitutes, when present, a noteworthy sign of plague in the rats.

As to the results of microscopical examination the bubo gives the best chance of recognizing plague bacilli in large numbers. Not only so, but the value of the bubo as an aid in the microscopical diagnosis of plague is increased by the presence in at least 50 per cent of those examined of the characteristic involution forms.

As to the relative value of diagnosis of the macroscopical and microscopical methods of diagnosis, the results of tests carried out for the purpose of comparison made it manifest that the naked eye is markedly superior to the microscopical method as an aid in diagnosis, and as the result of their experience the committee states that they are

prepared to make a diagnosis of plague on the strength of the macroscopical appearances alone, even though the results of cutaneous inoculation and culture are negative and the animal shows marked signs of putrefaction.

The value of the method of cutaneous inoculation of guinea pigs was examined. It would appear to fail in only about 2 per cent of fresh and about 10 per cent of putrid rats.

The bacilli found in naturally infected rats were fully virulent; 62 per cent of the inoculated animals died of acute plague in five days or less.

The rats examined were divided into two series. Series I comprised 200 plague rats (100 *Mus rattus* and 100 *Mus decumanus*) from those examined during the off season, from July to December, 1905, when sporadic cases only were occurring in rats and in men. All these rats were in a fresh condition—they showed no obvious signs of putrefaction. Series II consisted of 4,000 rats from those obtained during the early period of the epizootic—from the beginning of January to the middle of February, 1906.

Characteristic appearances in plague-infested rats recognizable by naked-eye examination.—Rigor mortis, the report states, is fairly often present in plague rats, and is somewhat characteristic, the limbs projecting stiffly in a distinctive manner from the body. It may persist, even when putrefaction has begun, in the internal organs. It was noted in 26.5 per cent of the rats in Series I.

Subcutaneous congestion is not infrequently a well-marked feature. It may be general, but in some cases is limited to the neighborhood of the bubo. In Series I it was present in 30.5 per cent of the total; in Series II a note was made of its presence in 69 per cent, it was well marked in 7 per cent, and was absent in 23 per cent of the rats. A peculiar purplish-red appearance of the muscles exposed by reflecting the skin of the thorax and abdomen is obviously due to the presence of congested vessels and, combined with the reddish-pink color of the subcutaneous tissue, presents an appearance which arouses a strong suspicion of plague at the commencement of the examination.

Emaciation was very rarely observed and is pronounced to be certainly not typical of plague. In a rat showing emaciation, and having lesions such as abscesses or septic lung conditions, the chances are greatly against the case being plague.

Subcutaneous hemorrhages were noted in 40.5 per cent of the rats in Series I. In 18.5 per cent the hemorrhages were situated in the submaxillary region, and were associated with the occurrence of a bubo in this region, while in 8 per cent subcutaneous hemorrhages were noted in the submaxillary region, although the bubo was in another situation or occasionally absent altogether. The general statement is made that when present these hemorrhages are most frequently to be found in the submaxillary region, depending doubtless upon the fact that hemorrhages are seen generally in the neighborhood of buboes and that buboes in rats are most often found in the neck. The next common situation for these hemorrhages is in the region of the flank. In young and medium sized rats especially they may be very widespread. They were not observed in any rat which was not plague infected. They rarely occurred in guinea pigs infected either experimentally or naturally.

A general edema of the subcutaneous tissue is a feature rarely met with in plague rats. When edema is present it is usually limited to the region of the bubo. In Series I cervical edema was present in 10 per cent of the cases. This contrasts with what was found in experimentally infected guinea pigs, in which general subcutaneous edema was a very characteristic feature.

Changes in the lymphatic glands—Buboes.—If a dissection is made of a healthy rat the only glands which are large enough to be easily seen are those forming the crescent embracing the salivary glands in the submaxillary region, and the elongated retroperitoneal glands on each side of the middle line in the lower part of the abdomen. For the sake of brevity the latter are referred to as "pelvic" glands.

In a septicemic plague rat the glands in any region of the body may be enlarged and congested. Even when a primary bubo is present, secondarily enlarged glands may be found in a different situation. Thus the inguinal glands are not infrequently slightly swollen and congested, and may be surrounded by a characteristic radiating appearance due to an injection of the blood vessels leading to and from the glands. Enlarged glands of this nature must be sharply distinguished from primary buboes. In the description the use of the word bubo is restricted to mean a primary bubo and not these secondary glands.

The Austrian plague commission in their account of the pathology of the lymphatic system in human plague make a distinction between primary buboes of the second order, i. e., glands in the neighborhood of the primary bubo which have been directly infected from it, and secondary buboes which derive their infection from the blood when a septicemia supervenes. In rats it is occasionally found that both the inguinal and pelvic glands are converted into primary buboes, the latter having obviously been infected by way of the lymphatics from the inguinal buboes. Such a lesion conforms to the description of a primary bubo of the second order.

Occasionally the primary bubo is seen in the first stage of enlargement and congestion, showing hemorrhagic points when cut across. It may be distinguished from a secondary gland by the surrounding infiltration, with perhaps hemorrhages in the subcutaneous tissue overlying it. Infiltration in the neighborhood of the bubo, extending into the subcutaneous tissue, is indeed a highly characteristic feature of a bubo in any stage of its development. A localized subcutaneous edema is sometimes observed. The presence of subcutaneous hemorrhages in the proximity of the bubo may often be noticed, and these are frequently associated with marked congestion of the surrounding tissues.

A bubo feels hard when cut across, though it has not the tough consistence of a normal gland. The contents of the latter are not easily squeezed out by pressure, whereas in a bubo the substance of the gland is readily broken down by slight pressure with the forceps.

Occurrence and distribution of buboes in 4,000 rats in Series II.

	Number.	Per cent.
Buboes in single situation only	2,923	73.05
Multiple buboes	467	11.67
Bubo absent	610	15.25

Buboes in single situation.

	Number.	Per cent.
Neck	2,194	75
Axilla	440	15.1
Groin	178	6.1
Pelvis	111	3.8

Frequency of various combinations of buboes in 467 rats with multiple buboes in Series II.

	Per cent.
Groin and axilla	32.3
Neck and axilla	28.2
Neck and groin	12.6
Neck, axilla, and groin	7.4
Groin and pelvis	7.1
Groin, pelvis, and axilla	4.9
Neck and pelvis	2.9
Axilla and pelvis	1.29
Neck, groin, and pelvis	1.07
Neck, axilla, groin, and pelvis	1.07
Neck, axilla, and pelvis	.85

Of the rats with multiple buboes, 54.5 per cent had bubo in the neck.

The typical appearance of a bubo on section is that of necrosis affecting first the medullary portion of the gland, and gradually spreading outward so that ultimately the glands are converted into a mass of necrotic tissue inclosed within the capsule. The central portion has consequently a gray appearance, or in a somewhat later stage contains a yellowish cheesy material. Rarely, in a still more advanced stage, the center has broken down into a rather dry—still more rarely a liquid—purulent material. Buboes with greenish liquid pus are not typical of plague, and those examined specially proved not to be plague.

At times but little surrounding congestion of the tissues is found and the bubo itself may have a yellowish-white color. Such a tissue offers a greater resistence than a normal gland when cut across. Microscopical examination of a bubo of this character reveals the presence of swarms of plague bacilli. Occasionally when a suspicious gland is cut across, a creamy fluid exudes which on microscopical examination is found to consist of degenerated leucocytes, cellular débris, and masses of plague bacilli.

The committee remarks that there is generally little difficulty in recognizing buboes, because they are usually relatively large in size and cause a prominent swelling. In the submaxillary region several buboes may be fused into a large mass. In certain instances, however, the existence of a bubo in the neck may easily be overlooked, for the

reason that there is not much apparent swelling even when the neck glands are exposed. The glands in this region should always be arranged in their natural relations, and cognizance taken of the slightest asymmetry. Any suspicious gland should be dissected out and cut across in several directions. The cut surface may show appearances suggestive of necrotic change, and if so, a smear should be prepared for microscopical examination. The committee made it a practice to cut into the neck glands of every rat examined. A bubo in the neck is sometimes readily found by probing with forceps in the region of the glands. Here it may be detected as a hard nodule like a pea.

Enlarged and congested glands in the groin and axilla should be incised and examined in the same way—a yellowish center, if only the size of a pinhead, being indicative that such a gland is a primary bubo.

Axillary buboes may readily be passed over when small, and especially if they are flattened and lie parallel to the inner surface of the arm under the insertion of the pectoral muscle into the humerus. A routine practice should therefore be adopted of cutting through these muscles into the axilla.

It is mentioned that a common and marked feature of a bubo when examined microscopically is the presence of more or less numerous involution forms. Although secondarily enlarged glands may contain numerous bacilli, these typically have the normal bipolar appearance.

The liver may show all degrees of fatty change. The term fatty, it is explained, is used in reference to the naked eye appearance only, which strongly suggests an excess of fat. Microscopically, however, the appearance is found to be due to necrosis of the liver tissue. In the early stages the lobules are clearly demarcated and this, combined with the yellowish appearance of the parts affected contrasting with the reddish color of the congested areas, constitutes a characteristic picture which is described by the term "mottling." In some instances an extreme degree of fatty change is seen. In such a case the liver has a pink tinge, its surface presenting a uniformly smooth appearance and showing no sign of any division into lobules. The whole organ gives the impression of being modeled in wax with the upper surface peculiarly dome-like and the edges sharply defined. It has lost the normal tough resilient consistence, so that it pits on pressure and somewhat easily cracks on bilateral pressure, especially when putrefaction has begun. This condition was not seen in fresh rats other than those which were plague infected, but in putrid rats an appearance very similar to it, or even indistinguishable from it, is rarely encountered when plague can, with certainty, be excluded.

Another condition frequently met with in the liver, and one of the greatest importance in diagnosis, is the occurrence of small necrotic foci scattered over its surface and throughout its substance. The condition is spoken of as "granular" liver. The gray or whitish granules are most easily observed on the surface; they are typically of the size of a pin's point and give the surface of the organ a stippled appearance as if dusted over with gray pepper. They are invariably discrete and in this respect contrast with the mottled liver in which there is no well-defined margin to any of the affected areas. They

may be so small that only the closest scrutiny of an experienced observer will detect them. When larger, the granules are of a yellow color and vary somewhat in size. When well marked and closely set together, they are always uniformly scattered throughout the liver substance, but if faintly marked and very few they may be confined to one lobe or to the edges only of the lobes. In some instances the necrosis assumes the appearance of a delicate gray network enclosing in its meshes the lobules, which appear reddish from the presence of congested vessels. In a typical speciment the granules are not raised above the surface of the liver. Very exceptionally this does happen.

This granular condition of the liver is fairly often met with in experimentally infected rats which die about forty-eight hours after inoculation. The longer the interval between inoculation and death the better marked is the granulation. In a few instances rats killed on the eighteenth day showed coarse granulation of the liver with very few plague bacilli present in the smears. With regard to the frequency of its occurrence, it was noted in 58 per cent of the rats in Series II. It is occasionally found in a liver which shows fatty changes. Even in putrid rats the granules may be recognized as gray points standing out on a black background.

Other pathological conditions met with in the liver in plague rats may be said to be neither constant nor characteristic. Hemorrhages under Glisson's capsule are seen relatively seldom. Enlargement and congestion of the liver, which some writers seem to consider noteworthy signs, are pronounced to be of very little value.

The spleen of a plague rat is typically of firm consistence with a molded appearance, so that it lies over the stomach in its natural relation to that organ instead of collapsing like a soft normal spleen. Granules or nodules, the size of a millet seed, may be very well marked and may be confluent. Sometimes a relatively large wedge-shaped portion of the spleen is converted into a cheesy mass, in which plague bacilli can be found. A false appearance of granulation is often seen in normal spleens, and is doubtless due to the Malpighian bodies showing through the semitransparent capsule. Apart from this a nodular condition was never seen by the committee except in a plague rat. Analysis of the records of 200 plague rats examined during December, 1905, showed that 111 of the livers were granular (55.5 per cent), while the spleen was granular in 9 (4.5 per cent). In very rare instances the spleen contains granules, although none are to be found in the liver.

Apparent enlargement and congestion of the spleen are of little importance for purposes of diagnosis. The spleen, especially in *Mus decumanus*, is often much enlarged, although the other organs are apparently normal; such a spleen is usually soft and flabby.

The kidneys and the suprarenal capsules are often congested. Minute subcapsular hemorrhages are fairly often present, viz, in 8.5 per cent of Series I. The kidneys frequently show fatty changes, sometimes appearing quite yellow. A granular condition of the organ is an extreme rarity, although occasionally it has been noted.

The stomach and the intestines usually show no characteristic change. The latter may be acutely congested, but subserous hemorrhages are rarely present, contrasting in this respect with plague

guinea pigs, in which they are a common and striking feature. Hemorrhages are somewhat rarely seen under the peritoneal coat of the stomach.

Abundant peritoneal effusion is a rare occurrence, though slight effusion may be seen, the serous surface having a moist look.

In the pleuræ and lungs hemorrhages occur fairly often, but were not seen in the parietal layer of the pleuræ.

The presence of pleural effusion is pronounced to be a very characteristic feature and of great value in diagnosis. The effusion is typically quite clear and may be so abundant that when the sternum and portions of the ribs are reflected the heart and lungs appear to be floating in a bath of straw-colored fluid which overflows, forming a pool in the axilla. It may sometimes be blood stained. In Series I it occurred in 73.5 per cent of the rats, while in 9 per cent it was abundant. In Series II its presence was observed in 72 per cent, it was noted as being abundant in 6.9 per cent, and it was absent in 28 per cent of the rats.

The lungs vary considerably in appearance and as a rule present nothing characteristic. They may exhibit a patchy congestion, but in some cases they appear quite pale. Compared with guinea pigs granules in the lungs of rats rarely occur, only 2.5 per cent of the rats showing them in Series I.

An interesting feature somewhat rarely met with in plague rats is a pneumonia which is decidedly lobar in character. In the cases examined microscopical examination of the lungs revealed very numerous plague-like bacilli (which were verified by culture in some of the cases), although relatively few or no bacilli were seen in the other organs. In two a submaxillary bubo was present, but the condition of the others leaves little doubt that they were instances of a typical primary pneumonia. Pneumonic lungs were observed in all stages, including typical red and gray hepatisation and even apparent resolution. Portions of consolidated lungs sank when placed in water.

The pericardium fairly often contains a clear fluid and epicardial hemorrhages occasionally are seen. The vessels on the surface of the heart frequently have an injected appearance. The walls are relaxed with the right cavities usually engorged with blood and the left empty.

The value of certain characteristic post-mortem features in the diagnosis of plague rats, including those which have undergone putrefaction.—A recapitulation is given of what are considered the most important post-mortem features for purposes of diagnosis.

The presence of a typical bubo is the most important sign of plague in rats.

The next important sign is the condition described as granular liver. This condition, in the experience of the committee, is not met with in rats other than those that are plague infected. The spleen is pronounced to be a much less important organ for diagnostic purposes than the liver; in this respect, it is said, the latter takes the place of the spleen in guinea pigs.

Hemorrhages, both subcutaneous and in the organs, are very suggestive features. They occurred somewhere in no less than 54 per cent of the rats in Series I. It is noted that subcutaneous hemorrhages constitute a most important sign of plague in rats.

Again, an abundant clear pleural effusion goes a long way of itself toward establishing a diagnosis of plague.

In putrid rats at least three of these signs may persist, and when recognized are of the greatest assistance, viz., a bubo, granular liver, and pleural effusion.

Frequency of occurrence of certain characteristic post-mortem features in the rats included in Series I.

Post-mortem appearance or lesion.	100 Mus rattus.	100 Mus decumanus	Percentage of total.
Rigor mortis	27	26	26.5
Subcutaneous congestion (including submaxillary)	22	39	30.5
Subcutaneous hemorrhages	44	37	40.5
Submaxillary hemorrhages with bubo	17	20	18.5
Submaxillary hemorrhages (bubo absent or in another situation)	7	9	8
Cervical edema	9	11	10
Fatty liver	59	50	54.5
Granules in the lungs	1	4	2.5
Granules in the kidneys	0	1	.5
Pleural effusion	73	56	64.5
Abundant effusion (included in above)	11	7	9
Hemorrhages in lungs and pleurae	16	32	24
Hemorrhages in kidneys and suprarenals	5	12	8.5
Hemorrhages in epicardium	2	5	3.5
Hemorrhages in stomach	4	0	2
Hemorrhages in intestines	0	1	.5

The occurrence of plague-like diseases among rats.—It is stated that during sixteen months' continuous rat examination in Bombay, involving the scrutiny of 150,000 animals, of which 19,000 were infected with plague, no disease of the rat was met with which caused any material difficulty in diagnosis.

Results of microscopical examination.—The importance of the results obtained from an analysis of this method of examination relates chiefly to the question of diagnosis.

For staining, carbol-thionin blue was used invariably in the routine examinations. This, it is stated, has a certain value as a differential stain in that plague bacilli appear more faintly colored than adventitious organisms. It brings out to advantage, it is said, the typical bipolar appearance of *Bacillus pestis*. Very rarely the bacilli in the organs assume the form of a small cocco-bacillus closely resembling the organism of fowl cholera and causing some doubt as to their real nature.

With regard to the presence of involution forms, 56.6 per cent of the buboes in Series I showed them, while in the same number of spleen preparations examined they were found in only 12 per cent. In the spleen they occur perhaps most frequently in association with putrefactive organisms. They were not observed in the heart blood.

Sometimes in rats which gave evidence of a relatively chronic form of plague, with well-marked granules in the liver, the bacilli were not uniformly distributed over the preparation, but were present in the form of characteristic clumps. Clumps of bacilli were seen in 9.5 per cent of the spleen smears in Series I. They rarely occurred in the heart blood, having been seen once only in this series. When in clumps the bipolar appearance was much less often observed than when the organisms were uniformly distributed in the smear, the contents of the bacilli usually appearing very finely and uniformly granular.

It is noted that the general value of the method of microscopical examination is sufficiently indicated by the fact that in 75 per cent of the total rats in Series I numerous plague bacilli were seen either in the heart blood, spleen, or bubo of each rat, or, if not very numerous in the bubo, involution forms were present.

As to the comparative value of the three tissues usually examined, there can be, it is declared, no doubt that the bubo gives a better chance of finding plague bacilli than the spleen and the spleen than the heart blood. Thus out of 150 rats with buboes in Series I numerous *Bacillus pestis* were noted in 104 preparations of the buboes, 70 preparations of spleens, and only 27 preparations of the heart blood. Even in a very putrid rat the bubo may show many plague bacilli, frequently with involution forms in addition, but with relatively much fewer putrefactive organisms than in the smears of the spleen or of the heart blood. In a suspicious bubo showing no plague bacilli the presence of degenerated leucocytes and cellular débris serves materially to strengthen the suspicion of plague.

The relative value of the methods of naked-eye and microscopical examination in the diagnosis of rats suspected of being plague infected.—It is stated that there is no room for doubt that for purposes of diagnosis naked-eye examination by a competent observer is more satisfactory than microscopical examination alone. In a single instance only was a plague rat diagnosed by microscopical examination which the observers of the post-mortem appearances failed to recognize, i. e., 0.7 per cent of the total number of plague rats. On the other hand, 6 rats with plague bacilli in the spleen smear were overlooked by the microscopists, and in 7 rats no plague bacilli were found microscopically in any of the organs, i. e., 13 rats were missed out of a total of 131, viz., nearly 10 per cent. In isolated cases both methods, it is added, must be employed, but results are said to clearly show that the omission of the routine microscopical examination of every rat in an investigation conducted on a large scale does not necessarily impair the accuracy of the work, while the saving of labor is of course very great. The investigation shows that the chief difficulty which is encountered in diagnosis by either of the methods arises from putrefactive changes masking the characteristic appearances in the organs.

Diagnosis by the cutaneous method of inoculation.—In nearly every case the rats examined were dead when brought to the laboratory and showed varying degrees of freshness or, in some cases, of putrefaction. The material inoculated was derived from 123 fresh rats and from 27 putrid rats. The majority of the rats belonged to the species *Mus decumanus*.

The technique adopted is described as follows, together with the effects which follow inoculation of plague material into the skin of a guinea pig: An area of skin about 1 inch square of the guinea pig's abdomen is shaved with a sharp razor, no water or soap being used. The use of soap is avoided in shaving the skin, as there is good reason to believe that the chances of the guinea pig dying acutely are thereby greatly diminished. The epidermis is partly removed in shaving, so that a raw, slightly bleeding surface is exposed. Pieces of the organ or organs selected for the test are then removed with sterile scissors and forceps, and rubbed with some vigor by means of the forceps

over the shaved area. This procedure is adhered to however putrid the material may be.

In the employment of the cutaneous method as a confirmatory test for rats diagnosed as plague or for rats suspected of being plague infected, the importance is pointed out of the bubo, if present, being rubbed in. It has been shown above that plague bacilli are more often found, and when present are more numerous, in the bubo of a plague-infected rat than in any other tissue.

From the point of view of early diagnosis by the cutaneous method special attention is directed in the report to the appearance of a reaction at the site of inoculation and the existence of enlarged inguinal glands.

The general experience of the committee was that the cutaneous reaction is the earliest symptom, usually appearing about twelve hours after inoculation. If the disease is acute, the inguinal glands can be felt to be enlarged thirty-six hours after inoculation, while in chronic cases the glands may be palpable only after the lapse of several days. A cutaneous reaction and the presence of inguinal buboes are outstanding features when the inoculation proves successful. Another symptom equally important is the loss of weight which occurs as the result of infection. When death takes place very acutely, the animal may even gain slightly in weight; otherwise there is a varying loss of weight, depending upon the acuteness of the disease. The average daily loss of weight was greatest in the case of guinea pigs which died on the fifth day. An early decrease in weight is shown to give valuable indication that infection by plague bacilli is in progress. A striking contrast is afforded in the case of guinea pigs in which infection has failed; the animal steadily gains in weight from the beginning and the skin abrasions rapidly heal.

The value of the cutaneous test for plague is especially manifest in those cases where the material inoculated is derived from rats which have undergone putrefaction. It is remarked that inoculation by this method apparently failed to produce infection in only three cases out of 27 putrid rats presenting appearances strongly suggestive of plague; i. e., it apparently fails in 10 per cent of putrid rats. It is therefore concluded that the cutaneous method gives an excellent chance of diagnosing plague even in rats far advanced in putrefaction.

Transmission of plague by feeding rats with infected material.—As a result of careful investigation the committee concludes that it is possible to infect wild rats of Bombay with plague by feeding them with the viscera of dead plague rats, 21.4 per cent being found susceptible to this method of infection. Bombay rats show a greater immunity to infection by feeding than rats of the same species which have not been subjected to a plague epizootic.

A series of experiments was also done with *Mus rattus* caught in the Punjab. Of these rats 67.8 per cent were susceptible. In this series a considerably larger dose of infected material was given.

The investigators infected a large number, 38 per cent, of wild Bombay rats by feeding them on the whole carcasses of their plague-infected comrades. No difference as regards the post-mortem appearances, or the distribution of the primary bubo, was found between rats infected in this way and rats infected by feeding on soft viscera.

The general pathological lesions found in all rats infected by feeding are, in the main, the same as those found in rats naturally in-

fected. There are, however, two striking differences: First, the common site of the primary bubo in naturally infected plague rats was in the neck, no mesenteric bubo having been seen out of 5,000 post-mortems, but in the case of fed rats the common site was the mesentery; second, in the case of naturally infected rats the stomachs and intestines showed no marked pathological change, while in the case of fed rats well marked pathological lesions were found in the intestines.

It would appear that in nature intestinal infection rarely or never takes place and that in consequence rats do not become infected by eating the carcasses of their comrades.

A large series of rats were fed on the urine of plague cases. None of these contracted the disease.

Significance of the locality of the primary bubo in animals infected with plague in nature.—The importance of the primary bubo as an indication of the path of infection is insisted upon in the report.

Bombay wild rats were inoculated with a virulent culture of the plague bacillus. Next day the rats were placed separately in flea-proof cages, and a number of fleas were put in with them. On the death of the rats the fleas collected from those rats which showed a marked plague septicemia were placed in a glass tube, one end of which was open, while the other end was closed with a single layer of fine muslin. About twenty fleas were put into each tube. The hair on a small area of a guinea pig's skin was removed. The fleas were now allowed to feed on this area through the muslin covering the end of the tube. They were given a morning and an evening meal, the tube being applied for from ten to fifteen minutes on each occasion. In some cases the feeding took place only on a single day, while in other instances the same fleas were fed daily for several days consecutively, the same area of skin, however, being used on each occasion. In the case of seventeen animals the relationship was shown between the area on which the fleas were fed, and through which the plague bacilli entered the skin, and the position of the primary bubo. A point of interest in these experiments was the presence in most cases and the complete absence in others of a marked local reaction at the site of feeding. Having then satisfied themselves that the primary bubo develops in those groups of glands which are in direct lymphatic connection with the area through which the plague bacillus enters the animal organism, the committee proceeded to inquire if, by a study of the relative distribution of the primary bubo, on the one hand, in animals naturally infected with plague, and, on the other hand, in animals artificially infected by different means, any evidence could be obtained which would point to any particular method as being the one by which infection takes place in nature.

Cervical buboes were found to preponderate, on the one hand, in naturally infected rats and in guinea pigs infected by being placed in plague-infected houses, and also in rats and guinea pigs artificially infected with fleas. In rats artificially infected by feeding mesenteric buboes were the most frequent, whereas in upword of 5,000 naturally infected rats in not a single case was a mesenteric bubo present. It is therefore concluded that rats in nature are not infected by feeding on plague-infected material, but probably by the agency of fleas.

The plague bacillus and the flea.—Observations were made on the transmission of plague by fleas, with special reference to the fate of the plague bacillus in the body of the rat flea (*Pulex cheopis*).

It was found that the average capacity of a rat flea's stomach may be approximately estimated to be 0.5 cubic millimeters; that a rat flea imbibing blood from a plague-infected rat might receive as many as 5,000 germs into its stomach; and that fleas feeding on a large proportion of plague-infected rats just before death imbibe some plague bacilli.

Fleas were fed on plague-infected rats until the death of these animals. They were afterwards fed on healthy animals. A number were dissected each day for twenty-three days. In a certain proportion abundant plague bacilli were found in the stomach contents up to the twelfth day and in one instance on the twentieth day. These observations were considered by the committee to be good evidence that multiplication of plague bacilli may take place in the flea's stomach.

The approximate proportion of fleas in the stomachs of which multiplication of plague bacilli takes place was determined, and it was shown that this proportion varies with the season of the year, being six times greater in the epidemic season than in the nonepidemic season.

Plague bacilli were present in the rectum and feces of fleas taken from plague rats, and such feces were found to be infective to guinea pigs both by cutaneous and by subcutaneous inoculation.

On rare occasions plague bacilli were found in the esophagus, but never in any other region of the body, such as the body cavity or salivary glands.

One series of experiments made during the epidemic plague season to test the duration of infectivity of rat fleas fed on septicemic rats' blood showed that these fleas could remain infective for at least ten days. This series was made in separate cages with a limited supply of fleas. A second series also made during the epidemic season, but in a single large cage in the presence of a large number of fleas, gave the time that fleas might remain infective as fifteen days. In a third series of experiments conducted under the same conditions as the second series, but during the nonepidemic season, the fleas remained infective for seven days only, and, further, far fewer (one-third instead of two-thirds) animals than in the second series contracted the disease.

A single rat flea may transmit the disease. Both male and female rat fleas can transmit the infection.

Twenty-seven experiments to transmit plague from animal to animal by means of cat fleas (*Pulex felis*) were made. None of these was successful. Thirty-eight experiments to transmit plague from animal to animal by means of human fleas (*Pulex irritans*) were made. Three were successful. Two experiments were made with *Ceratophyllus fasciatus;* both gave successful results.

Evidence was obtained to show that the bite of a healthy flea affords a sufficient avenue for infection by septicemic blood if it is spread upon the bitten part. No evidence was brought out in favor of infection by contaminated mouth parts or regurgitation from the stomach, but the possibility of infection by such means could not be excluded.

Natural occurrence of chronic plague in rats.—The characteristic feature of chronic rat plague is described as being the presence of circumscribed abscesses containing plague bacilli in rats caught alive, the animals usually showing no other lesions nor signs of ill health. No bacilli were seen on microscopical examination of the heart blood and of the spleen tissue in any of the rats. The bacilli in the great majority of the cases were virulent. Forty-five rats conforming to this description which were found during the year's investigation reported in 1907 in the Punjab were grouped into two classes, one group including those in which the lesions were situated in the abdominal viscera, and the other group including those in which the abscesses were found in regions occupied by peripheral lymphatic glands. Lesions of the viscera were found principally in the spleen and in the mesentery, while the submaxillary group were most frequently affected among the lymphatic glands. The peripheral type was observed chiefly during the decline of the epizootic, while the visceral type predominated in the offseason. In Bombay only one chronic plague rat was met with out of 17,000 plague-infected rats. In Kasel 9 per cent of all the rats which were proved plague infected had the chronic disease, while in Dhand the proportion was as high as 28 per cent. In the present state of knowledge the committee declare themselves unable to advance any adequate explanation of these facts. No direct evidence was found that chronic plague, as it occurs in the Punjab villages, possesses any significance in the seasonal recurrence among the rats of the infection in an acute form, nor was any evidence available which excludes this possibility.

Man as a host of the Indian rat flea.—It is shown that in the laboratory the rat flea, *Pulex cheopis*, will readily bite man. When very numerous it will bite man even in the presence of its natural host. The committee were able to keep this species of flea alive for more than three weeks by feeding it on man alone. In the course of some experiments in go-downs which were infested with *Pulex cheopis* alone, fleas in considerable numbers were often caught on the legs of men who had entered the go-downs for a short time. In a building in Bombay in which there had been a severe rat mortality, proved to be due to plague, rat fleas in large numbers were caught on the legs of men who entered some of the rooms in the building for a short time.

It is concluded, therefore, that the rat flea, *Pulex cheopis*, under certain circumstances, is attracted by man, and will readily bite and feed on him.

UNITED STATES.

[Reports to the Surgeon-General, Public Health and Marine-Hospital Service.]

Reports from San Francisco, Cal.—Status of plague—Plague-prevention work.

Passed Assistant Surgeon Blue reports:

December 4. One new case of plague to-day, a Russian. Total cases of plague to date, 110; deaths to date, 65.